

Marked Up Version of Page 22 Showing Changes Being Made

$10^7/\text{well}$) in complete RPMI medium are cultured for up to 72 h in the presence or absence of worm antigen or anti-CD3 and then the supernatants are assayed for cytokines and immunoglobulins. IFN- γ , TNF α and IgG2a characterize a Th1 response, whereas IL-4, IL-5, IgE and IgG1 typify a Th2 reaction. Also, serum can be assayed for cytokine and immunoglobulin concentrations. Furthermore, dispersed inflammatory leukocytes are examined by flow cytometry for Fc γ 3 expression on macrophages (Th1) and MHC Class II expression on B cells (Th2). Controls include serum, MLN and spleens from appropriate age-matched, littermate mice that hosted no parasite. Also, there are other markers of the Th1 vs Th2 responses.

A similar analysis can differentiate a human Th1 from a Th2 response. One examines inflamed tissue, isolated leukocytes from regions of inflammation and peripheral blood cells. Leukocytes are cultured in vitro alone or in the presence of parasite antigen or mitogens to stimulate cytokine release, and the cytokines are analyzed by, for example, ELISA. The specific pattern of cytokines released allows differentiation of Th1 from Th2 responses. IgG2 is generally indicative of a Th1 response, whereas IgE-, IgG1 or IgG2 are indicative of Th2 response.

Cytokine Detection by Flow Cytometry: Splenocytes, MLN or intestinal inflammatory cells in RPMI complete medium are placed into 24-well tissue culture plates at 2×10^6 cells/well. Cells are incubated 4-6h in the presence or absence of anti-CD3 or appropriate antigen with brefeldin A at 10 $\mu\text{g}/\text{ml}$. Brefeldin prevents exocytosis of proteins and promotes accumulation of the cytokine within the cell. For cytoplasmic cytokine detection, the cells are fixed in 2% paraformaldehyde at room temperature for 5 min following surface staining to distinguish cell subtypes. Cells are washed and re-suspended in 50 μl PBS 0.2% Saponin and 1 μg anti-cytokine antibody and incubated at room temperature for 20 minutes. Next, the cells are washed twice in Saponin and re-suspended in PBS/FCS. The specificity of the cytokine antibody staining is confirmed by pre-blocking the cells with an excess of un-conjugated antibody of the same isotype and cytokine specificity or by incubating the cells in the presence of recombinant cytokine. Phycoerythrin (PE)-labeled irrelevant antibody controls also are included to assess background staining. The cells are analyzed using flow cytometry.

Marked Up Version of Page 49 Showing Changes Being Made

absence of exposure to intestinal helminthic infections in childhood is an important environmental factor.

Several animal models have been developed to study MS in animals. These models include cuprizone-induced demyelination, infection with virus and Experimental Allergic Encephalomyelitis (EAE). Of these various models, EAE has been the most studied and has served for many years as an excellent animal model for studying the pathogenesis of MS.

EAE is an experimental autoimmune inflammatory demyelinating disease of the CNS and serves as an animal model to study demyelinating diseases of the CNS. It has three forms, which vary in clinical course and neuropathology: acute EAE, hyperacute EAE and chronic relapsing EAE. Acute EAE and hyperacute EAE are monophasic diseases, which resemble the human diseases acute disseminated encephalomyelitis and acute hemorrhagic leukoencephalitis, respectively. Chronic relapsing EAE has a relapsing course and is most similar to MS. EAE is induced by subcutaneous injection of central nervous tissue homogenate or antigens emulsified in complete Freund's adjuvant (CFA) into susceptible animals. Several antigens have been shown to be encephalitogenic, such as myelin basic protein (MBP) and myelin proteolipid protein (PLP).

Studies Using the EAE Model of Multiple Sclerosis

1. Schistosome ova injection significantly ameliorates the clinical course of PLP-induced EAE in SJL/J mice.

In the first experiments, two experimental protocols have been optimized to characterize the effect of schistosome ova injection on EAE. The two protocols are different only in the frequency of high or low dose immunization with schistosome ova. In Protocol #1, 6-8 week old female SJL/J mice were injected intraperitoneally with 10,000 schistosome ova fourteen days prior to EAE induction. Schistosome ova injection was repeated at day 4 prior to EAE induction using 5000 schistosome ova intraperitoneally and 5000 subcutaneously. This protocol had been shown to induce a very strong Th2 type immune response. On the day of EAE induction (day 0), the experimental animals were injected by subcutaneous tail base injection of 50 ug of PLP139-151 (HSLGKWGH^PDKF) (SEQ ID NO. 1) peptide in CFA containing 1 mg